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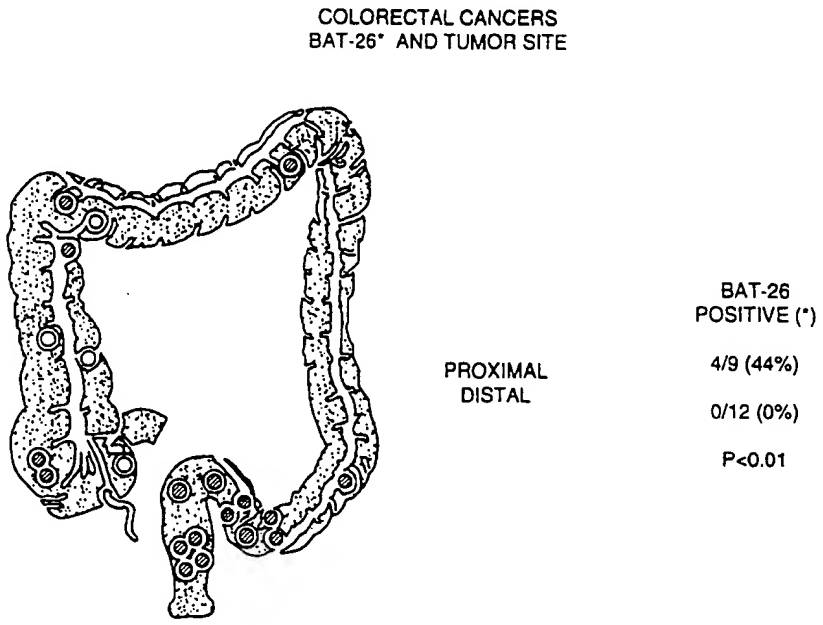

(57) Abstract

Methods of the invention comprise assays for markers indicative of cancer, precancer, and other diseases or disorders. Assays of the invention are preformed on heterogeneous samples obtained from patients by non-invasive or minimally-invasive methods. Such assays may be employed alone or in combination with other disease screening techniques.

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METHODS OF DETECTING COLORECTAL DISEASE

RELATED APPLICATIONS

This application claims the benefit of U.S. Application No. 60/134,711.

FIELD OF THE INVENTION

The invention relates to methods of detecting cancer, precancer, or other diseases or disorders comprising performing an assay to detect one or more nucleic acid markers effective for use alone or in combination with other testing techniques.

BACKGROUND OF THE INVENTION

Cancer is thought to arise from a multi-step process that typically involves multiple genetic mutations leading to uncontrolled cell growth. Many cancers are curable if detected early in their development. For example, colorectal cancers typically originate in the colonic epithelium, and are not extensively vascularized (and therefore not invasive) during early stages of development. The transition to a highly-vascularized, invasive and ultimately metastatic cancer commonly takes ten years or longer. If the presence of cancer is detected prior to extensive vascularization, surgical removal typically is an effective cure. However, colorectal cancer is often detected only upon manifestation of clinical symptoms, such as pain and bloody stool. Generally, such symptoms are present only when the disease is well established, and often after metastasis has occurred. Similarly, with the exception of the Pap smear for detection of pre-malignant cervical lesions, diagnostic screening methods for other types of cancer are best at detecting established disease.

Most diagnostic assays for cancer are invasive, or at least uncomfortable. Invasive procedures range from performing a tissue biopsy to surgery. Cancer screening procedures frequently result in significant patient discomfort. For example, magnetic resonance imaging requires confinement of the patient, and colonoscopy requires sedation. The discomfort associated with typical invasive screening methods reduces patient compliance with routine screening procedures.

Typically, patients with a significant family history of colorectal cancer, such as those with Hereditary Non-Polyposis Colorectal Cancer ("HNPCC"), will undergo genetic testing using known assays. Those who test positive will then typically undergo colonoscopy.

However, those patients who test negative generally undergo further testing (i.e., colonoscopy) less frequently, if at all, despite the fact that they are among an "at-risk" group. Typical testing regimens allow up to four years between colonoscopy tests for "at-risk" subjects. Furthermore, due to the expense and discomfort associated with colonoscopy testing, there is a significant non-compliant patient population who avoid testing altogether.

Even for patients who undergo invasive testing, effective detection of cancer often still presents problems. For example, using flexible sigmoidoscopy, it is only possible to detect cancers at the left side (distal or lower end) of the colon. Cancers developing at the right side (proximal or upper end) of the colon are generally undetected by this technique.

The problems of sensitivity and specificity are exaggerated in assays for the early detection of cancer because patient samples on which such early detection is performed typically contain relatively small amounts of cancerous cellular material in relation to non-cancerous cellular material. In many cases, patient samples are a heterogeneous mixture of large amounts of normal cells and small amounts of cancerous cells. A good example of such a heterogeneous sample is stool. The typical stool sample contains cells and cellular debris sloughed from the colonic epithelium, by-products of digestion, and bacteria. In its early stages, colorectal cancer is thought to affect only about 1% of colonic epithelial cells. Any attempt to detect nucleic acids from the 1% of affected cells in the heterogeneous background of the stool sample might give rise to very low sensitivities. Attempts to identify the presence of the indicia of cancer in other heterogeneous samples, such as sputum, pus, urine, nipple or aspirate presents similar problems.

Recently, a number of genetic mutations have been associated with diseases or disorder, of the colon. For example, alterations in the BAT-26 segment of the MSH2 mismatch repair gene, the p53 gene, the Kras oncogene, and the apc tumor suppressor gene are thought to be participants in a multi-step pathway leading to cancer. It has been suggested that mutations in those genes might be a basis for molecular screening assays for the early stages of certain types of cancer. See e.g., Sidransky, et al., Science, 256: 102-105 (1992). Attempts have been made to identify and use nucleic acid markers that are indicative of cancer. However, even when such markers are found, using them to screen patient samples, especially heterogeneous samples, has proven unsuccessful either due to an inability to obtain sufficient sample material, or due to the low sensitivity that results from measuring only a single marker. For example, simply obtaining adequate human DNA from one type of heterogeneous sample (stool) has proven difficult. See Villa, et al., Gastroenterol., 110: 1346-1353 (1996) (reporting that only 44.7% of all stool

specimens, and only 32.6% of stools from healthy individuals produced sufficient DNA for mutation analysis). Other reports in which adequate DNA has been obtained have reported low sensitivity in identifying a patient's disease status based upon a single cancer-associated mutation. See Eguchi, et al., Cancer, 77: 1707-1710 (1996) (using a p53 mutation as a marker for cancer).

Accordingly, there is a need in the art for high-sensitivity, high-specificity assays for the detection of molecular indicia of cancer, pre-cancer, and other diseases or disorders, especially in heterogeneous samples.

SUMMARY OF THE INVENTION

Methods of the invention address the problem of obtaining accurate results in an assay for indicia of cancer, precancer, or other diseases or disorders in a heterogeneous sample. These methods further provide enhanced detection techniques when used in combination with other invasive or non-invasive detection techniques.

The methods of the present invention exploit the discovery that mutations in the BAT-26 segment of the MSH2 mismatch repair gene are closely associated with inherited cancers (and pre-cancerous lesions). In particular, BAT-26 mutations are highly-associated with HNPCC (i.e., in greater than 90% of patients), making BAT-26 an ideal marker for screening assays to detect this colorectal cancer, or colorectal adenoma that may or may not develop into cancer.

For purposes of the present invention a mutation is a deletion, addition, substitution, rearrangement, or translocation in a nucleic acid. A loss of heterozygosity is a form of mutation in which all or a portion of one allele is deleted. Also for purposes of the present invention, the terms "markers", "targets", and "mutations" include nucleic acid (especially DNA) mutations (substitutions, additions, rearrangements, translocations, deletions, single nucleotide polymorphisms, etc.), as well as other nucleic acid indicia useful in methods of the invention. Such indicia include the amount of amplifiable nucleic acid in a sample, the length of nucleic acids in a sample, the ratio of long nucleic acids (greater than about 200 base pairs) to short nucleic acids (less than about 200 base pairs), and any other nucleic acid variations that differ between patients with cancer and disease-free patients. Also for purposes of the present invention, the terms "healthy" or "disease-free" are intended to mean a patient who does not have cancer, precancer, or other relevant diseases or disorders.

In one embodiment, the present invention relates to utilizing a DNA marker, preferably BAT-26, to screen for the presence of cancerous or precancerous lesions in patients. The patients

may have symptoms or a history by which they are considered to be part of an "at-risk" group, especially for inherited forms of colorectal cancer, particularly HNPCC.

Furthermore, BAT-26 mutations have been found to be associated with cancers located in the right-hand (proximal) side of the colon. Thus, the methods of the present invention contemplate utilizing a combinatorial testing approach to screen patients, wherein BAT-26 testing is used to screen the right side of the colon, and flexible sigmoidoscopy is utilized to screen the left hand (distal/lower) side of the colon. Such a testing methodology permits a far more thorough screen for cancerous and/or precancerous lesions than was previously possible using tests practiced in the art. Thus, in another embodiment, the present invention provides methods for detecting the presence of colorectal cancerous or precancerous lesions comprising (i) conducting in a sample obtained non-invasively or minimally-invasively from a patient an assay to identify a BAT-26 marker in the sample, and (ii) performing a flexible sigmoidoscopy on the patient.

The methods of the invention are useful for detecting diseases or disorders related to the colon including, but not limited to, cancer, pre-cancer and other diseases or disorders such as adenoma, polyp, inflammatory bowel disorder, inflammatory bowel syndrome, regional enteritis, granulomatous ileitis granulomatous ileocolitis, Crohn's Disease, ileitis, ileocolitis, jejunoileitis, granulomatous colitis, Yersinia enterocolitica enteritis, ulcerative colitis, psuedo-membraneous colitis, irritable bowel syndrome, diverticulosis, diverticulitis, intestinal parasites, infectious gastroenteritis, toxic gastroenteritis, and bacterial gastroenteritis.

The methods of the present invention also provide for the use of BAT-26 as a marker for detection of cancerous and precancerous lesions by analysis of heterogeneous samples (e.g. stool). Such methods comprise obtaining a representative sample of a stool voided by a patient and performing an assay on the sample to identify a BAT-26 marker in the sample.

Stool is a good example of a heterogeneous sample in which methods of the invention are especially useful. A typical stool sample contains patient nucleic acids, but also contains heterologous nucleic acids, proteins, and other cellular debris consistent with the lytic function of the various nucleases and proteinases found in the colon. Under normal circumstances, stool solidifies as is proceeds from the proximal colon to the distal colon. As the solidifying stool passes through the colon, colonic epithelial cells are sloughed onto the stool. If a patient has a developing neoplasia, cells from the neoplasia will also be sloughed into stool, and they (or their debris) will contain molecular indicia of disease (e.g., mutations or loss of heterozygosity). In

the early stages of development, nucleic acid indicative of a neoplasia comprise only about 1% of the nucleic acid in a voided stool. If a patient is left untreated, proportionately more disease-related nucleic acids are found in stool over time. Methods of the invention are useful for detecting early-stage lesions in heterogeneous samples such as stool. Methods of the invention result in a high degree of sensitivity and specificity for the detection of early-stage disease. Methods of the invention are especially useful in detecting, for example, adenomas in the colon. Adenomas are non-metastatic lesions that frequently have the potential to become cancer. If all adenomas in a patient are detected and removed, the probability of complete cure in a patient developing colorectal cancer is virtually zero.

In another preferred embodiment, methods of the invention comprise selecting one or more mutational events that are indicative of cancer, precancer, or other diseases or disorders, such that the combined informativeness of the one or more events meets or exceeds a predetermined or desired level of informativeness. The informativeness of any mutation or combination of mutations may be validated by an accepted invasive screening technique. For example, in methods to detect colorectal cancer, the informativeness of a molecular assay may be determined by identification of a lesion using colonoscopy.

A detailed description of certain preferred embodiments of the invention is provided below. Other embodiments of the invention are apparent upon review of the detailed description that follows.

DETAILED DESCRIPTION OF THE DRAWINGS

Figure 1 is a table showing the results of a clinical study of screening assays performed on 40 subjects using various markers, including BAT-26.

Figure 2 is a pictorial representation of the location of nine cancers located in the study described in Figure 1.

Figures 3 and 4 are tables showing the results of a clinical study of screening assays performed on 28 subjects using various markers, including BAT-26. Figure 5 depicts the DNA sequence of the BAT-26 locus, wherein each "n" corresponds to a nucleotide of unknown identity.

DETAILED DESCRIPTION OF THE INVENTION

Methods of the invention provide non-invasive or minimally-invasive assays for the detection of early stage cancer, precancer, or other diseases or disorders. Methods of the

invention are especially useful in detecting cancer or precancer in heterogeneous biological samples. Preferred methods comprise identifying in a patient sample one or more nucleic acid mutations(s) that provide high sensitivity and high specificity for detection of the indicia of cancer or precancer. Methods of the invention comprise identifying mutations having a known informativeness for cancer or precancer, or may be based upon validating selected mutations or assays to detect them with respect to a standard assay for cancer. Preferred methods comprise assays utilizing detection of BAT-26 mutations. By utilizing cancer or precancer markers having a high sensitivity/specificity for detecting the presence of cancer or precancer, methods of the invention provide improvements in non-invasive or minimally-invasive molecular screening assays. For purposes of the present invention, non-invasive or minimally-invasive indicates that specimens for analysis are obtained either from bodily fluids (e.g. stool, pus, sputum, blood aspirate, or lymph).

The invention will be exemplified with experiments to detect the presence of indicia of colorectal cancer or precancer in samples prepared from patient stool specimens. However, the skilled artisan recognizes that methods of the invention can be practiced using a variety of different samples in order to detect a variety of cancers, pre-cancers, and other diseases and disorders.

A reason that detection of colorectal cancer or precancer (e.g., an adenoma) is exemplified is that a stool specimen is a good example of a heterogeneous environment in which methods of the invention are especially useful (see above). Moreover, colonoscopy (and sigmoidoscopy, a related technique) is a well-known invasive standard that has a high sensitivity and high specificity (although high cost and low patient compliance) with which methods of the invention can be compared and validated.

Methods of the invention comprise screening a sample, such as one prepared from a stool specimen, for the presence of one or more marker(s) of cancer, precancer, or other diseases or disorders (e.g., a colorectal tumor or adenoma), such that the sensitivity of detection is between about 50% and about 100%, and the specificity of detection is between about 85% and about 100%. In a preferred embodiment, methods of the invention combine different types of assays in order to achieve an overall increase in sensitivity and specificity. Thus, methods of the invention comprise conducting an assay for a mutation known to be associated with cancer, precancer or another disease or disorder, and an assay for a quantity and/or length of DNA expected to occur in conjunction with the cancer, precancer, or other disease or disorder in order to obtain the

combined benefits of the sensitivity and specificity of both assays. Moreover, embedded within the concept of utilizing multiple nucleic acid analyses to detect a disease or disorder is the use of multiple genomic targets in each assay in order to provide further increases in sensitivity and specificity. However, as shown below, a single-marker assay is sufficient for practice of the invention.

The genomic targets and assay methods used according to the invention can vary depending upon the desired level of sensitivity and specificity, as well as the type of disease or disorder the detection of which is desired. Genomic targets (e.g., mutations) are selected based upon their known sensitivity or specificity or by determining a baseline sensitivity and specificity. In preferred embodiments, methods of the invention comprise the detection of a mutation at a single, informative locus. In other embodiments, assays for informative loci are combined in order to achieve improved sensitivity and specificity of detection relative to invasive techniques. Accordingly, methods of the invention contemplate a combination of assays selected from multiple mutation detection, quantitative polymerase chain reaction (i.e., to determine the amount of amplifiable DNA in a sample), sequence-specific hybrid capture, oligo-ligation, amplification refractory mutation system, single-stranded conformational polymorphism detection, sequencing, mismatch detection, and single base extension. Representative assays can be found in co-owned and co-pending U.S. Application No. 09/371,991, incorporated by reference herein. Target loci include chromosomes 1, 5, 8, 17, and 18, particularly chromosome 5q, chromosome 17p, chromosome 8p, chromosome 1q, and chromosome 18q. Preferred loci for use in methods of the invention include p53, apc, BAT-26, and others suspected to be predictive of certain diseases or disorders. The most preferred locus for use in methods of the invention is BAT-26.

Other genes are known to be associated with colorectal cancer, and their sensitivity and specificity are determined when not known in the literature by determining the percentage of tumors bearing the mutation, and the percentage of healthy specimens that bear the mutation from a sufficiently large and diverse population. This can be done empirically, or mathematically using algorithms that predict the likelihood of false positive and false negative screening results based upon data relating the presence of a mutation to the presence of cancer, pre-cancer or another disease or disorder. In the case of colorectal cancer, confirmation of a patient's clinical status can be accomplished by a standard test such as colonoscopy (which has a typical sensitivity of 95% and a typical specificity of 100%).

For the analysis of stool samples, preferred methods of the invention comprise obtaining at least a cross-section or circumferential portion of a voided stool as taught in U.S. patent numbers 5,741,650, and 5,952,178, both of which are incorporated by reference herein. While a cross-sectional or circumferential portion of stool is desirable, methods provided herein are conducted on random samples obtained from voided stool, which include smears or scrapings. Once obtained, the stool specimen is homogenized. A preferable buffer for homogenization is one that contains at least 16mM ethylenediaminetetraacetic acid (EDTA). However, as taught in co-pending, co-owned U.S. patent application serial number 60/122,177, incorporated by reference herein, it has been discovered that the use of at least 150mM EDTA greatly improves the yield of nucleic acid from stool. Thus, a preferred buffer for stool homogenization comprises phosphate buffered saline, 20-100 mM NaCl or KCl, at least 150mM EDTA, and optionally a detergent (such as SDS) and a proteinase (e.g., proteinase K).

After homogenization, nucleic acid is preferably isolated from the stool sample. Isolation or extraction of nucleic acid is not required in all methods of the invention, as certain detection techniques can be adequately performed in homogenized stool without isolation of nucleic acids. In a preferred embodiment, however, homogenized stool is spun to create a supernatant containing nucleic acids, proteins, lipids, and other cellular debris. The supernatant is treated with a detergent and proteinase to degrade protein, and the nucleic acid is phenol-chloroform extracted. The extracted nucleic acids are then precipitated with alcohol. Other techniques can be used to isolate nucleic acid from the sample. Such techniques include hybrid capture, and amplification directly from the homogenized stool. Nucleic acids can be purified and/or isolated to the extent required by the screening assay to be employed.

Nucleic acids to be analyzed are chosen based upon known or suspected relationships between specific mutations and cancer, precancer, or other diseases or disorders. If desired, sequence-specific hybrid capture is used to isolate specific nucleic acids from the sample. Target nucleic acids may be analyzed by any method of the art. Examples of preferred methods include enumerative analysis of the loss of heterozygosity as taught in U.S. patent number 5,670,325, incorporated by reference herein. Enumerative methods do not require knowledge of the sequence of a mutant nucleic acid. Rather such methods determine that there has been an alteration (deletion, substitution, addition, rearrangement, or other mutation) in a wild-type nucleic acid. The investigated loci are chosen based upon the likelihood of an alteration being associated with cancer, precancer, or another disease or disorder. Enumerative methods compare

the number in a sample of a wild-type nucleic acid known not to be altered in a specific disease or disorder with the number of a wild-type nucleic acid known or suspected to be altered in a specific disease or disorder. A statistically-significant difference in the two numbers indicates a positive screen.

Mutations in target nucleic acids may also be measured by single base extension techniques to identify a single nucleotide variant indicative of cancer or precancer. Preferably, single base extension assay are cycled as taught in co-owned, co-pending U.S. patent application serial number 09/067,212, incorporated by reference herein. Briefly, cycled single base extension reactions comprise annealing a nucleic acid primer immediately 5' to a region containing a single base to be detected. The single base to be detected represents a marker for mutation. The mutation may be a single point mutation or may be a larger mutation for which the single base is a marker. Two separate reactions are conducted. In the first reaction, primer is annealed to target, and labeled (preferably ^{32}P) nucleic acids complementary to non-wild type (e.g. mutants indicative of disease) variants at the single base to be detected, and unlabeled dideoxy nucleic acids complementary to the wild-type base are combined. Primer extension is stopped the first time a wild-type (dideoxy) base is added to the primer. Presence of label in the extended primer is indicative of the presence of a mutation. A second tube, the positive control contains labeled nucleic acid complementary to the wild-type base in the presence of the primer. A DNA polymerase, such as Sequenase™ (Amersham), is used for primer extension. In a preferred embodiment, a thermostable polymerase, such as Taq or thermal sequenase is used to allow more efficient cycling. Once an extension reaction is completed, the first and second probes bound to target nucleic acids are dissociated by heating the reaction mixture above the melting temperature of the hybrids. The reaction mixture is then cooled below the melting temperature of the hybrids and additional primer is permitted to associate with target nucleic acids for another round of extension reactions. In a preferred embodiment, 10 to 50 cycles of extension reactions are conducted. In a most preferred embodiment, 30 cycles of extension reactions are conducted. After completion of all cycles, extension products are isolated and detected. In alternative embodiments, chain-terminating methods other than dideoxy nucleotides may be used. For example, chain termination occurs when no additional bases are available for incorporation at the next available nucleotide on the primer.

Methods of the invention are also useful for screening populations of patients in order to identify characteristics in population samples that are indicative of cancer or adenoma. For

example, methods of the invention comprise high sensitivity, high specificity screening of populations of patients in order to correlate nucleic acid mutations or polymorphic variants present in a subset of patient samples with the presence of disease in those patients. Thus, methods of the invention comprise detecting genomic variations in patient samples, correlating those variations with confirmed disease, and using the variations associated with confirmed disease as a diagnostic screen for the disease in subsequent patient samples. Such methods preferably are performed on pooled samples, such as stool samples, from identified populations of patients (e.g., diseased, healthy). Such methods are preferably based upon variations in single nucleotide polymorphic loci. The sensitivity and specificity of detecting variants in those loci as a function of disease is determined. Those loci that predict disease at predefined levels of sensitivity and specificity are selected for use in screening assays for unknown patient samples.

Methods of the invention are useful not only for detecting cancer or precancer, but also for detecting other colonic diseases or disorders that may be correlated with specific nucleic acid markers including, but not limited to, adenoma, polyp, inflammatory bowel disorder, inflammatory bowel syndrome, regional enteritis, granulomatous ileitis granulomatous ileocolitis, Crohn's Disease, ileitis, ileocolitis, jejunoileitis, granulomatous colitis, Yersinia enterocolitica enteritis, ulcerative colitis, psuedo-membraneous colitis, irritable bowel syndrome, diverticulosis, diverticulitis, intestinal parasites, infectious gastroenteritis, toxic gastroenteritis, and bacterial gastroenteritis.

The following examples provide specific exemplification of the concepts discussed above. The assays exemplified below are for purposes of illustration.

Example 1 – Clinical Study of Cancer Detection Using BAT-26 Marker

Stool specimens were collected from 40 individuals who presented at the Mayo Clinic (Rochester, MN) with symptoms or history indicating that a colonoscopy should be performed. Each stool sample was frozen. Immediately after providing a stool sample, all individuals were given a colonoscopy in order to determine their disease status. Colonoscopy, an invasive test requiring sedation of the patient, has a sensitivity approaching 95%, and a specificity of nearly 100% for the diagnosis of colonic neoplasia. Based upon the colonoscopy results and subsequent histological analysis of biopsy samples taken during colonoscopy, individuals were placed into one of three groups: normal, cancer, and adenoma. An adenoma, or polyp, is considered clinically relevant if it has a diameter of 1 cm or greater. Thus, all individuals in the adenoma group had a polyp of at least 1 cm in diameter. Patients in the cancer group had tumors

diagnosed as cancer, and the disease-free individuals were those for whom colonoscopy showed no sign of cancer or adenoma. Based upon the colonoscopy results, 21 patients were diagnosed with cancer, 9 patients were diagnosed with an adenoma greater than 1 cm, and 10 patients were free of cancer or adenoma.

Multiple mutation analysis was then performed, on a blinded basis (i.e., scientists performing the assays did not know the results of colonoscopy or histology), on each sample. Each frozen stool specimen, weighing from 7-33 grams, was thawed, homogenized in 500 mM Tris, 16 mM EDTA, and 10 mM NaCl, pH 9.0, at a volume to mass ratio of about 3:1. Samples were then rehomogenized in the same buffer to a final volume-to-mass ratio of 20:1, and spun in glass macro beads at 2356 x g. The supernatant was collected and treated with SDS and proteinase k. The DNA was then phenol-chloroform extracted and precipitated with alcohol. The precipitate was suspended in 10 mM Tris and 1 mM EDTA (1 x TE), pH 7.4. Finally, the DNA was treated with Rnase.

Human DNA was isolated from the precipitate by sequence-specific hybrid capture. Biotynilated probes against portions of the p53, K-ras, and apc genes were used.

A 10 ul aliquot of each probe (20 pmol/capture) was added to a suspension containing 300 ul DNA in the presence of 310ul 6M GITC buffer for 2 hours at room temperature. Hybrid complexes were isolated using streptavidin-coated beads (Dynal). After washing, probe-bead complexes were suspended at 25° C for 1 hour in 0.1x TE buffer, pH7.4. The suspension was then heated for 4 minutes at 85° C, and the beads were removed.

Captured DNA was then amplified using PCR, essentially as described in U.S. Patent No. 4,683,202, incorporated by reference herein.

Samples were heated to 94° C for 5 minutes, and then 40 cycles were conducted between 94° C, 60° C, and 72° C (1 minute each), followed by one cycle at 72° C for 5 minutes.

Amplified nucleic acid samples were then run on an electophoretic gel and size differences in the amplified PCR products were observed to detect mutant samples.

As shown in Figure 1, four out of nine cancers found had BAT-26 mutations.

As shown in Figure 2, all nine cancers were found in varying parts of the colon, but only the right-sided cancers had BAT-26 mutations.

Example 2 – Diagnostic Assay Using BAT-26

The BAT-26 mismatch repair locus (Figure 5) was used to assess the same 40 samples described above. Deletions in BAT-26 have been associated with colorectal cancer or adenomas.

Samples were prepared as described above. A primer was hybridized to the portion of the BAT-26 locus immediately upstream of the poly-A tract, which consists of 26 adenosines (nucleotides 195-221).. Unlabeled deoxythymidine, a mixture of labeled and unlabeled deoxycytosine, and unlabeled dideoxyadenine were added along with polymerase. The primer was extended through the poly-A region. The labeled and unlabelled cytosine was extended for the next three bases (nucleotides 222-224, all guanines in the intact sequence) such that label was incorporated into each extended primer. After the poly-A tract and the three guanines, there exist two thymidines in the intact sequence. Thus, the dideoxyadenosine stops primer extension by addition at the end of a primer that has been extended through the poly-A and triguanine regions. Strands were separated, and the length of the strands was observed on a polyacrylamide gel to detect deletions in the poly-A tract. The results are presented below in Table A:

TABLE A

Patient Status	Diagnosis By Colonoscopy	Diagnosis By BAT-26 Detection	Sensitivity of BAT-26 Detection	Specificity of BAT-26 Detection
Cancer/Adenoma	21/9	4/0	19%/0%	100%/0%

As shown above, BAT-26 alone did not provide the high sensitivity achieved using multiple mutation or quantitation alone, but showed high sensitivity in comparison with other single locus detection assays. Moreover, as shown below, BAT-26 in combination with the other techniques described above produced an overall increase in sensitivity and specificity.

Example 3 - Cumulative Effects of Kras, Multiple Mutation, Quantitation, and BAT-26

The results obtained above for Kras, multiple mutation analysis, quantitation, and BAT-26 were combined to determine the cumulative effects of using combinations of those techniques in order to produce increased sensitivity and specificity in a non-invasive assay for cancer or precancer. The results are summarized below in Table B:

TABLE B

Assay Combination	Kras and Quantitation and BAT-26	Quantitation and BAT-26	Multiple Mutation and Quantitation and BAT-26
Sensitivity for Detection of Cancer/Adenoma	80%/56%	80%/56%	90%/78%
Specificity for Detection of Cancer/Adenoma	100%	100%	100%

As shown in the summary above, the combination of multiple mutation analysis, quantitative PCR, and BAT-26 produced a sensitivity approaching that of colonoscopy. A combination of multiple mutation analysis and quantitation alone also produces very high sensitivities. All assays resulted in a specificity of 100% (no false positive results), which is comparable to colonoscopy.

The foregoing experiments show that even a single high-sensitivity/high specificity non-invasive or minimally-invasive assay produces diagnostic results that are superior to non-invasive/minimally-invasive techniques of the art, and approach results observed with the recognized standard invasive diagnostic procedure (colonoscopy). Moreover, a non-invasive assay utilizing more than one high-sensitivity/high-specificity technique results in diagnostic accuracy approaching 100%. As such, methods of the invention provide a significant improvement in the ability to perform accurate non-invasive diagnosis of cancer.

Example 4 – Clinical Study of Cancer Detection Using BAT-26 Marker

The methods described above in Example 1 were followed using stool specimens collected from 28 individuals at the Mayo Clinic (Rochester, MN) with symptoms or history indicating that a colonoscopy should be performed. The results are shown in Figures 3 and 4, and demonstrated that the study found two of eight cancers with BAT-26 mutations.

Claims:

What is claimed is:

1. A method for detecting the presence of a colonic disease or disorder in a patient comprising:

conducting an assay to detect a mutation in a BAT-26 locus in a nucleic acid present in a sample of patient tissue or body fluid; and

detecting a colonic disease or disorder as a positive assay for said mutation.

2. The method of claim 1, wherein said assay comprises the steps of:

(a) contacting the sample with an oligonucleotide primer that is complementary to a portion of the BAT-26 locus immediately upstream of the polyA tract at the BAT-26 locus;

(b) extending said primer in the presence of dTTP, thereby to form a primer extension product;

(c) extending the primer extension product in the presence of a labeled nucleotide complementary to a nucleotide base downstream from the polyA tract at the BAT-26 locus, wherein said labeled nucleotide is not dTTP; and

(d) comparing the size of the labeled extension product obtained in step (c) to a standard, wherein a labeled extension product that is smaller or larger than the standard is indicative of a mutation in the BAT-26 locus.

3. The method of claim 1, wherein said sample is a stool sample.

4. The method of claim 1 wherein said disease or disorder is cancer.

5. The method of claim 4, wherein said cancer is colorectal cancer.

6. The method of claim 5, wherein the colorectal cancer is Hereditary Non-Polyposis Colorectal Cancer.

7. The method of claim 3 wherein said disease or disorder is selected from the group consisting of pre-cancer, adenoma, polyp, inflammatory bowel disorder, inflammatory bowel syndrome, regional enteritis, granulomatous ileitis granulomatous ileocolitis, Crohn's Disease, ileitis, ileocolitis, jejunoileitis, granulomatous colitis, Yersinia enterocolitica enteritis, ulcerative

colitis, psuedo-membraneous colitis, irritable bowel syndrome, diverticulosis, diverticulitis, intestinal parasites, infectious gastroenteritis, toxic gastroenteritis, and bacterial gastroenteritis.

8. The method of claim 3, wherein said stool sample is homogenized.

9. The method of claim 3, wherein said sample is selected from the group consisting of urine, blood, sputum, cerebrospinal fluid, pus, and aspirate.

10. A method of determining a site of colonic disease or disorder in a patient, the method comprising the steps of:

conducting an assay to identify in a patient tissue or body fluid sample a mutation in the BAT-26 locus; and

determining that said disease or disorder is present in the proximal colon if said assay is positive for a mutation.

11. The method of claim 10 further comprising the steps of:

performing flexible sigmoidoscopy on the patient to identify a colonic lesion or polyp; and

determining that said disease or disorder is present in the distal colon if a colonic lesion or polyp is identified.

12. The method of claim 10 wherein said disease or disorder is cancer.

13. The method of claim 12, wherein said cancer is colorectal cancer.

14. The method of claim 13, wherein the colorectal cancer is Hereditary Non-Polyposis Colorectal Cancer.

15. The method of claim 10 wherein said disease or disorder is selected from the group consisting of pre-cancer, adenoma, polyp, inflammatory bowel disorder, inflammatory bowel syndrome, regional enteritis, granulomatous ileitis granulomatous ileocolitis, Crohn's Disease, ileitis, ileocolitis, jejunoileitis, granulomatous colitis, Yersinia enterocolitica enteritis, ulcerative colitis, psuedo-membraneous colitis, irritable bowel syndrome, diverticulosis, diverticulitis, intestinal parasites, infectious gastroenteritis, toxic gastroenteritis, and bacterial gastroenteritis.

16. The method of claim 10 wherein said sample is stool.

17. The method of claim 16, wherein said stool sample is homogenized.

18. The method of claim 10, wherein said sample is selected from the group consisting of urine, blood, sputum, cerebrospinal fluid, pus, and aspirate.

19. A method for detecting the presence of a colonic disease or disorder in a patient comprising the steps of

conducting a first assay to detect a mutation in a BAT-26 locus in a nucleic acid present in a sample of patient tissue or body fluid

conducting a second assay to identify a mutation in second locus in the nucleic acid , the second locus being selected from the group consisting of a p53 locus, an apc locus, and a Kras locus; and

detecting colonic disease or disorder as a positive first or second assay.

20. A method for confirming the presence of a colonic disease or disorder in a patient comprising the steps of:

determining the presence of a colonic disease or disorder according to the method of claim 1;

performing colonoscopy on the patient, and

confirming a colonic disease or disorder as a lesion or polyp detectable by colonoscopy.

21. A method of determining a patient at risk of developing Hereditary Non-Polyposis Colorectal Cancer, comprising the steps of:

detecting the presence of a colonic disease or disorder in the patient in accordance with method of claim 3, said colonic disease or disorder being an adenoma, and

determining the patient at risk of developing Hereditary Non-Polyposis Colorectal Cancer as a patient having an adenoma.

	KRAS	APC	P53	BAT26	Q	RESULTS
CANCER N=21	*(4)* A46 A59 A5 A54	*(5)* A74 A54 A76 A57 A16	*(3)* A74 A82 A49	*(4)* A9 A5 A6 C943	*(14)* A58 A59 A46 A5 A61 A54 A74 A76 A77 A81 A82 A51 A33 A16	SENSITIVITY 90% (19 OF 21)
ADENOMA N=9	*(0)*	*(3)* N466 C899 N767	*(0)*	*(0)*	*(5)* C835 C902 N767 C935 N159	SENSITIVITY 78% (7 OF 9)
NORMAL N=10	*(0)*	*(0)*	*(0)*	*(0)*	*(0)*	SPECIFICITY 100% (10 OF 10)

NOTE: RESULTS ARE UNALTERED IF KRAS EXCLUDED

FIG. 1

COLORECTAL CANCERS
BAT-26* AND TUMOR SITE

	BAT-26 POSITIVE (*)
PROXIMAL	4/9 (44%)
DISTAL	0/12 (0%)
	P<0.01

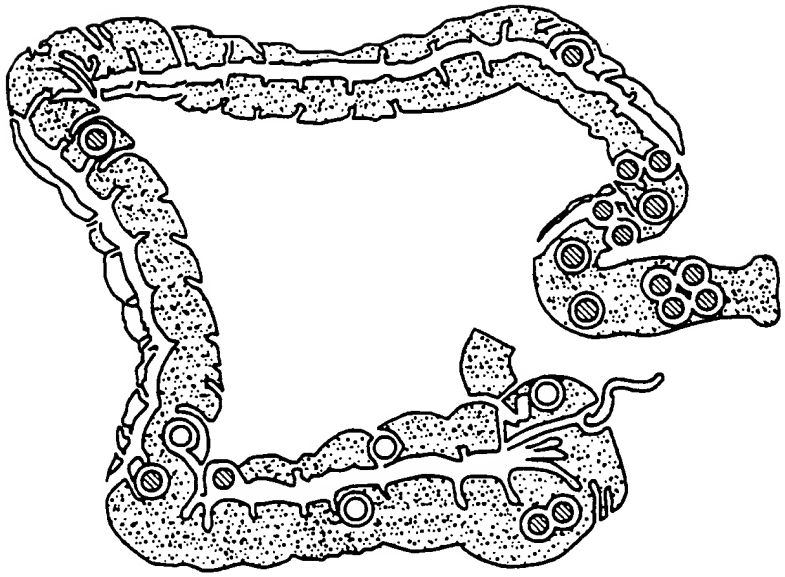

FIG. 2

RESULTS
EXCLUDING KRAS

	APC	P53	BAT26	IQ	RESULTS
CANCER N=8	*(2)* A74 A16	*(1)* A82	*(2)* C943 C1227	*(5)* A33 A82 A74 A61	SENSITIVITY: 89% (7 OF 8)
ADENOMA N=2	*(0)*	*(0)*	*(0)*	*(1)* N739	SENSITIVITY: 50% (1 OF 2)
NORMAL N=18	*(0)*	*(0)*	*(0)*	*(0)*	SPECIFICITY: 100% (18 OF 18)

FIG. 3

RESULTS
INCLUDING KRAS

	KRAS	APC	P53	BAT26	IQ	RESULTS
CANCER N=8	*(2)* A46 A74	*(2)* A74 A16	*(1)* A82	*(2)* C943 C1227	*(5)* A33 A82 A74 A61	SENSITIVITY 100% (8 OF 8)
ADENOMA N=2	*(1)* N772	*(0)*	*(0)*	*(0)*	*(1)* N739	SENSITIVITY 100% (2 OF 2)
NORMAL N=18	*(2)* N726 N778	*(0)*	*(0)*	*(0)*	*(0)*	SPECIFICITY 89% (16 OF 18)

FIG. 4

<223> BAT-26, WHEREIN EACH "n" CORRESPONDS TO A NUCLEOTIDE OF UNKNOWN IDENTITY

<400> 37
ccagtggtat agaaatcttc gatttttaaa ttcttaattt taggttgcag tttcatcact 60
gtctgcggta atcaagtttt tagaactctt atcagatgat tccaactttg gacagtttga 120
actgactact tttgacttca gccagtatat gaaattggat attgcagcag tcagagccct 180
taaccttttt caggtaaaaa aaaaaaaaaa aaaaaaaaaa agggttaaaa atgttgattg 240
gttaannnnn nnngacagat agtgaagaag gcttagaaag gagctaaaag agttcgacat 300
caatattaga caag 314

FIG. 5